REMARKS

Claims 1-5, 7-21 and 23-77 are pending following entry of the amendments above. Claim 1 has been amended to incorporate the recitations of claim 6, which has been cancelled. Claim 19 has been amended to incorporate the subject matter of claim 22 (now cancelled) to distinguish the subject matter of claim 19 from claim 1. Claim 35 has been amended to more clearly recite a Markush group. New dependent claims 55-77 have been added to provide a more complete claim set with respect to independent claims 32 and 37. The recitations of new dependent claims 55-77 are similar to original claims 3, 4, 7, 8, 10-14,15 and 35. Applicants submit that neither the claim amendments nor the new claims present new matter, and Applicants respectfully request entry thereof.

Applicants note with appreciation the indication in the Office Action that the subject matter of claims 24, 25, 41, 42, 45, 47, 53 and 54 is allowable, and that the subject matter of claims 23, 46 and 52 would be allowable if rewritten to be in independent form and to address the rejections under § 112, second paragraph. The remaining issues raised in the Office Action will be addressed below.

I. Indefiniteness.

Claims 23, 46 and 52 stand rejected as indefinite under § 112, second paragraph for lack of sufficient antecedent basis for particular recitations in the claims. In particular, claim 23 is rejected for insufficient antecedent basis for "the oligosaccharides." Claim 23 has been amended to omit the word "the" to recite "oligosaccharides." Claims 46 and 52 stand rejected for insufficient antecedent basis for the recitation of "said quantifying step." Claims 46 and 52 have been amended to recite "said determining step."

Applicants submit that these claim amendments address the rejections under § 112, second paragraph and respectfully request withdrawal thereof.

II. Rejection under 35 U.S.C. § 102(b).

Claims 1-7, 9, 10, 14-21, 26-30, 32 and 33 stand rejected under 35 U.S.C. §102(b) as anticipated by the article Chester et al., "Increased Urinary Excretion of a Glycogen-Derived Tetrasaccharide in Heterozygotes with Glycogen Storage Diseases Type II and III" from the Lancet (referred to as "Lancet" in the Office Action). The Applicants respectfully disagree with this rejection.

The Lancet reference describes a single observational study performed with a small number of patients (nine) with Pompe disease (glycogen storage disease type II [GSDII]) and seventeen clinically healthy family members. Urinary excretion of $(Glc)_4$ was compared between the two groups and with the published range for $(Glc)_4$ excretion in healthy subjects. Two of the nine GSDII patients had $(Glc)_4$ concentrations in urine in or close to the range for non-affected family members (first and fifth GSDII subjects, 9.9 and 17.7 mg/24 h urine), whereas one unaffected sibling excreted $(Glc)_4$ into urine at a higher level (40.3 mg/24 h urine) than six out of the nine GSDII subjects.

Thus, the preliminary and crude results presented in the Lancet reference do not disclose to those of ordinary skill in the art the use of $(Glc)_4$ as a biomarker to screen subjects for Pompe disease as recited by claims 1-7, 9, 10, 14-21 and 26-30. The person of ordinary skill in this art would view the data presented in Lancet with skepticism and would recognize the preliminary nature of this study. The Lancet article reference falls far short of providing an enabling disclosure to those of ordinary skill in the art of the use of $(Glc)_4$ to screen subjects with Pompe disease as presently claimed. In contrast, the present inventors have provided a validated biomarker and have shown through rigorous evaluation that $(Glc)_4$ is a biomarker to identify subjects with Pompe disease as well as to monitor the clinical condition of subjects with Pompe disease.

At most, the Lancet reference suggests a trend toward increased urinary excretion of $(Glc)_4$ in patients with Pompe disease and heterozygous carriers as compared with normal subjects. There would have been considerable doubt in the mind of the ordinary skill worker whether $(Glc)_4$ could be used to screen subjects with

Pompe disease based on the Lancet reference. In particular, the Lancet reference indicates that $(Glc)_4$ would not be a suitable biomarker as the limited data presented in the reference suggest that $(Glc)_4$ cannot distinguish between unaffected carriers (heterozygotes) and subjects with Pompe disease.

Further, the present claims recite a method of screening a subject for Pompe disease by determining the concentration of $(Glc)_4$ in a biological sample, and comparing the concentration with a reference value, "wherein the detection of $(Glc)_4$ in the biological sample at more than the reference value identifies the subject as affected with Pompe disease." The Lancet reference does not disclose a screening method at all or, more particularly, a screening method using a "reference value," wherein a $(Glc)_4$ concentration greater than the reference value identifies the subject with Pompe disease. The Lancet reference looked at urinary $(Glc)_4$ concentrations in carriers and patients with Pompe disease and compared them with the published range for a normal population. They observed that both unaffected carriers and patients with Pompe disease had elevated urinary $(Glc)_4$. Lancet does not screen a single subject or identify a single unknown subject as affected with Pompe disease. Moreover, Lancet does not employ a "reference value" to identify subjects with Pompe disease as compared with unaffected subjects.

In summary, the Lancet reference does not disclose a method of using $(Glc)_4$ to screen subjects for Pompe disease as recited by claims 1-7, 9, 10, 14-21, 26-30, 32 and 33.

A. Claims 14, 18, 19-21, 26 and 30.

With particular respect to the rejection of claim 14, Applicants note that the Lancet article evaluated urine samples, and does not disclose or suggest the use of a "cell or tissue sample" as recited by claim 14.

Likewise, the Lancet article does not disclose or suggest the use of $(Glc)_4$ as a screening assay in conjunction with additional diagnostic testing as recited by claims 18 and 30.

With respect to claims 15-17, as discussed above, Lancet does not disclose or suggest the use of a reference value to identify subjects with Pompe disease.

Claim 19 has been amended to recite "determining the concentration of hexose tetrasaccharide $(Glc)_4$ in a biological sample taken from the subject by tandem mass spectrometry," thereby mooting this rejection. Claims 20 and 21, which depend from claim 19, incorporate the features of claim 19. These claims will be addressed below in connection with the rejections under § 103 in section III(E) below.

Further, the Lancet article does not disclose or suggest a semi-quantitative method as recited in claim 26.

B. Claims 32 and 33.

Claims 32 and 33 recite a method of "monitoring the clinical condition of a subject with Pompe disease" wherein the detection of $(Glc)_4$ in a biological sample from the subject "is indicative of the clinical condition of the subject." Clearly, the Lancet reference is completely silent as to a method of monitoring the clinical condition of a subject with Pompe disease. In particular, nowhere is there any disclosure or suggestion in the Lancet reference to use $(Glc)_4$ to monitor the condition of a subject with Pompe disease, *e.g.*, to monitor the course of the disease over time, to assess the response of the subject to treatment and/or to determine when to commence or re-initiate treatment of the subject.

C. The claims are novel over the Lancet reference.

For the reasons set forth above, Applicants submit that the subject matter of claims 1-7, 9, 10, 14-21, 26-30, 32 and 33 is novel over the Lancet reference, and respectfully request that the outstanding rejection under § 102(b) be withdrawn.

III. Rejections under § 103(a).

Claims 8, 11, 12, 13, 22, 31, 34-40, 43, 44 and 48-51 stand rejected under 35 U.S.C. § 103(a) as unpatentable for obviousness over the Lancet publication alone or in view of Hua et al., Macri, Kikuchi et al. and/or Millington et al. The individual obviousness rejections will be addressed below.

A. Lancet.

Claims 8 and 31 stand reject as unpatentable for obviousness over the Lancet reference. Claims 8 and 31 recite methods of screening neonatal subjects for Pompe disease by detecting $(Glc)_4$. The Examiner states that "Lancet teaches the invention substantially as claimed with the exception of expressly teaching that the subject of Glc_4 is a neonate. However, Lancet does teach that Glc_4 can be detected in prenatal fetuses as well as patients that range in age from 5-20 years" (Office Action, page 3, point 5). Applicants respectfully submit that the Lancet reference has been misconstrued.

The Applicants respectfully point out that Lancet does not disclose or suggest that $(Glc)_4$ can be detected in neonatal urine (or any other biological sample from a neonate). The Lancet reference certainly does not disclose or suggest that $(Glc)_4$ can serve as a biomarker for screening neonatal subjects for Pompe disease as recited by claims 8 and 31.

Applicants assume that the statement in Lancet relied on for the present rejection is:

> In one family (VIII) the mother of a GSD II patient became pregnant for a second time. Prenatal enzymatic diagnosis revealed an affected fetus, which was aborted. The level of $(Glc)_4$ in her urine was essentially the same when tested before she became pregnant and a few days before and a few days after termination of pregnancy (Lancet, page 995, col. 1, lines 5-10; *emphasis added*).

The quoted statement is referring to the detection of $(Glc)_4$ in the urine of the pregnant woman, not the fetus. There are no teachings in Lancet whatsoever regarding detection of $(Glc)_4$ in embryonic or neonatal subjects. If the Examiner is

relying on another portion of the Lancet article and this rejection is maintained, it is respectfully requested that the relevant portion be pointed out in the next Office Action so that Applicants can address it in their response.

The presently claimed methods of screening a neonatal subject for Pompe disease using $(Glc)_4$ would not have been at all obvious to those skilled in the art at the time of invention. It would not have been obvious that the effects of the metabolic disorder (*i.e.*, insufficient or absent lysosomal acid α-glucosidase activity) would have manifested in a neonatal subject and would result in $(Glc)_4$ accumulation at sufficient levels by the neonatal period. Certainly, such a suggestion is not provided by Lancet or any of the other cited references.

In view of the foregoing discussion, Applicants submit that the subject matter of claims 8 and 31 is nonobvious over the Lancet reference, and respectfully request that the rejection under §103 over Lancet be withdrawn.

B. <u>Neonatal Blood Samples</u>.

Claims 11 and 12 stand rejected over Lancet further in view of Hua et al. and U.S. 5,252,489 (Macri). Claims 11 and 12 recite methods of screening a neonate for Pompe disease using a blood sample and a dried blood spot, respectively. The Examiner has applied Lancet as described above. The Examiner further states that Hua et al. teaches that "it is known in the art to use neonatal blood samples when testing for markers indicative of lysosomal storage disorder" (Office Action, page 4, lines 10-11). Further, the Examiner discusses the advantages of testing neonatal blood for markers of metabolic disorders, such as PKU. The Examiner applies Macri as teaching the advantages of dried samples. The Examiner concludes that "it would have been obvious to one of ordinary skill in the art to modify the screening method of Lancet to include dried blood samples as taught by both Hua and Macri." This rejection is respectfully traversed below.

As discussed in the preceding section, the Lancet reference does not disclose or suggest that $(Glc)_4$ is a biomarker for Pompe disease during the neonatal period.

Likewise, no suggestion or motivation for the use of $(Glc)_4$ to screen neonatal subjects for Pompe disease is to be found in the cited Hua et al. or Macri references.

Furthermore, the Office Action discusses the advantages and desirability of using blood samples and dried blood spots to screen for $(Glc)_4$ accumulation. However, none of the cited references taken alone or in any combination disclose or suggest that $(Glc)_4$ would correlate with Pompe disease during the neonatal period or that it would accumulate to detectable levels in blood during the neonatal period. Indeed, as discussed in the specification (page 4, lines 10-11 and page 28, lines 5-10), as far as the inventors are aware, it has not previously been disclosed that $(Glc)_4$ is present in blood (or blood-derived fluids) for subjects of any age. It would not have been at all obvious that $(Glc)_4$ would accumulate in blood based on reports of $(Glc)_4$ excretion in urine.

Turning to Hua et al., this reference discloses the use of LAMP-1 and LAMP-2, lysosomal membrane proteins, as non-specific markers in plasma for lysosomal storage disorders. Hua et al. acknowledge the shortcomings of these markers because of the high degree of false positives (*see, e.g.*, final paragraph of discussion). More importantly, however, this reference does not in any way provide a suggestion or motivation to measure $(Glc)_4$ in blood or dried blood spots to screen for Pompe disease in neonates as recited by claims 11 and 12. Further, with respect to dried blood spots, neither Hua et al. nor Macri discloses or suggests that $(Glc)_4$ would be sufficiently abundant or stable for detection in dried blood spots (*e.g.*, typically a 1/8 inch punch of a dried 50 μl sample).

In summary, Lancet, Hua et al. and Macri, taken alone or in any combination, fail to disclose or suggest a method of screening a neonatal subject for Pompe disease by detecting $(Glc)_4$ in blood or dried blood spots. Accordingly, Applicants respectfully submit that the subject matter of claims 11 and 12 is nonobvious over these references, and request that the outstanding obviousness rejection be withdrawn.

C. Dried Urine Samples.

The Office Action rejects claim 13 as unpatentable for obviousness over Lancet further in view of Macri. Claim 13 recites a method of screening neonatal subjects for Pompe disease by detecting $(Glc)_4$ in a dried urine sample. The Lancet and Macri references have been discussed in the preceding sections. Lancet does not disclose or suggest the use of $(Glc)_4$ as a biomarker for Pompe disease during the neonatal period. Further, this reference does not disclose or suggest the use of a dried sample, such as a dried urine sample, or that $(Glc)_4$ is stable when dried. These deficiencies are not remedied by Macri. Neither Lancet nor Macri provide any suggestion that: (a) $(Glc)_4$ is a biomarker of Pompe disease during the neonatal period, (b) that $(Glc)_4$ accumulates during the neonatal period, (c) $(Glc)_4$ accumulates to sufficient levels in the urine of subjects with Pompe disease during the neonatal period to be useful in screening neonatal subjects for the disease, and (d) $(Glc)_4$is sufficiently abundant and stable to be detected in a dried urine sample.

Thus, in view of the foregoing, it is submitted that Lancet and Macri, alone or in combination, do not render the subject matter of claim 13 obvious. Accordingly, Applicants request withdrawal of the outstanding obviousness rejection over these references.

D. Monitoring Clinical Condition of Subjects Undergoing Treatment for Pompe Disease.

The Office Action has rejected claims 34-38 under §103(a) as unpatentable for obviousness over Lancet in view of Kikuchi et al. The Office Action states that it would have been obvious to provide some form of treatment for those suffering from Pompe disease, and further states that "[a]s enzyme replacement therapy is the latest and most promising therapy for this disorder it would be expected that this treatment would be utilized" (Office Action, page 6, lines 10-11). The Office Action continues that "any patient undergoing treatment for a disease as serious as Pompe disease would most certainly be under the care of a physician and would be monitored carefully for any changes in their condition so that the therapeutic regimen

could be adjusted accordingly" (Office Action, page 6, lines 12-15). Applicants address this rejection below.

Claims 34-36 are directed to methods of monitoring the clinical condition of a subject, where the subject is undergoing treatment for Pompe disease. As discussed in more detail in section II above, the Lancet reference does not disclose or suggest a method of monitoring the condition of a Pompe disease patient by detecting $(Glc)_4$. Moreover, Kikuchi et al. only concerns the use of enzyme replacement therapy, which Applicants agree is useful in the treatment of Pompe disease. Kikuchi et al. does not provide any teachings regarding the use of $(Glc)_4$ to monitor the clinical condition of a subject undergoing enzyme replacement, or any other, therapy.

With respect to claims 37 and 38, these claims are drawn to methods of assessing the efficacy of a therapeutic regime in a subject with Pompe disease by detecting $(Glc)_4$. Applicants agree that it would be desirable to be able to monitor subjects with Pompe disease undergoing treatment so as to assess the efficacy of the treatment and/or to make adjustments in the treatment. It is not at all obvious, however, based on the Lancet reference or Kikuchi et al., taken alone or in combination, that $(Glc)_4$ would be suitable for use in assessing the therapeutic efficacy of a treatment regime in a subject with Pompe disease. The Lancet publication does not assess therapeutic efficacy or in any other way monitor subjects with Pompe disease by detection of $(Glc)_4$. This suggestion is not provided by the Kikuchi et al. reference either, which simply describes the use of enzyme replacement therapy for treating Pompe disease.

Moreover, for the sake of argument, even if it was known that $(Glc)_4$ was elevated in subjects with Pompe disease, it would not have been obvious to one of ordinary skill in the art that $(Glc)_4$ could serve as a biomarker to monitor the clinical condition of a subject with Pompe disease or to assess therapeutic efficacy. The identification of appropriate biomarkers that track the clinical condition of a subject is not a straight-forward or routine matter. Many biomarkers that are elevated in a particular disease state fail to track the clinical condition of the subject. For example,

some biomarkers provide a "yes/no" indication of whether a disease state is present, but do not correlate with the severity of the disease. As another problem, some biomarkers do not fluctuate with the disease state. In particular, the biomarker may not be able to track amelioration in the clinical condition (*i.e.*, once the subject's condition has deteriorated, the biomarker cannot "rebound" or "recover" in response to improvement in the subject's condition). As another concern, many biomarkers do not change quickly enough to be useful in tracking the clinical condition of the subject.

The inventors have demonstrated that $(Glc)_4$ is a useful biomarker to monitor the clinical condition of a patient with Pompe disease over time and to assess the efficacy of therapy. In clinical trials, $(Glc)_4$ has been used to monitor the clinical condition of patients undergoing therapy for Pompe disease. In these studies, the $(Glc)_4$ biomarker tracks the clinical condition of the subject and is an early indicator of change in clinical condition. This outcome would in no way have been obvious in view of the Lancet reference alone or in combination with Kikuchi et al.

Thus, the observations in Lancet that some patients with Pompe disease and unaffected heterozygotes have an elevation in urinary excretion of $(Glc)_4$ in no way renders obvious the claimed methods of monitoring the clinical condition of a subject or assessing the efficacy of a therapeutic regime in a subject with Pompe disease as recited by claims 34-38. Accordingly, it is submitted that the subject matter of claims 34-38 is nonobvious over the art, and Applicants request that the rejection for obviousness over Lancet in view of Kikuchi et al. be withdrawn.

E. <u>Tandem Mass Spectrometry</u>.

Claims 22, 43, 44 and 48 stand rejected as unpatentable for obviousness over Lancet further in view of Millington et al. Claim 22 has been cancelled.
Claims 19-21 will also be discussed in connection with this rejection. Claims 19-21 recite a method of screening a subject for Pompe disease by detecting $(Glc)_4$ using tandem mass spectrometry ("TMS"). Claims 43, 44 and 48 recite a method of determining the concentration of $(Glc)_4$ in a biological sample by TMS. The

Examiner concedes that the Lancet reference "fails to teach that Glc_4 is quantified by tandem mass spectrometry" (Office Action, page 7, lines 1-2). The Examiner states that Millington et al. teach the advantages of using TMS. This rejection is addressed below.

With respect to claims 19-21, as addressed above, the Lancet reference does not provide any suggestion to use $(Glc)_4$ as a biomarker to screen subjects for Pompe disease. In the absence of any such suggestion, one of ordinary skill in the art would have had no motivation to modify the detection methods of the Lancet reference to apply TMS to detect $(Glc)_4$ in a screening method for Pompe disease as recited by claims 19-21.

Furthermore, the Millington et al. reference discusses the advantages of TMS methodology. However, this reference fails to disclose or suggest in any way that TMS can be applied to detect $(Glc)_4$, for example, to detect $(Glc)_4$ in a screening method for Pompe disease (claims 19-21) or to determine the concentration of $(Glc)_4$ in a biological sample taken from a subject (claims 43, 44 and 48). At most; Millington et al. would suggest the desirability of the TMS methodology, but does not render the identification of a method of detecting $(Glc)_4$ by TMS obvious.

The development of a TMS protocol to measure a new analyte is a substantial undertaking. Various issues must be considered to adapt the technology to a new analyte such as the abundance of the analyte in the starting material, the presence of biological isomers, homologues or other interfering molecules as well as the presence of other substances that cause "ion suppression" and interfere with detection and measurement of the analyte. Derivatization methods can be evaluated to facilitate detection of the analyte and/or to move the signal away from an interfering substance. Further, separation techniques can be used to remove interfering substances and/or to increase the concentration of the analyte. In sum, even if one of ordinary skill in the art would have desired to detect $(Glc)_4$ by TMS, the development of a method to detect $(Glc)_4$ by TMS would not have been at all obvious.

Applicants therefore submit that the subject matter of claims 22, 43, 44 and 48 is both novel and nonobvious over the cited references, and respectfully request withdrawal of the outstanding rejection under § 103.

F. Neonatal Screening Methods using TMS Analysis of Blood Samples.

Claims 39, 40, 49 and 50 stand rejected as unpatentable for obviousness over Lancet in view of Hua, Macri and Millington. This rejection is respectfully traversed below.

The rejected claims are drawn to methods of screening a neonatal subject for Pompe disease by detecting $(Glc)_4$ using TMS in a dried blood spot (claims 39 and 40) or a method of determining the concentration of $(Glc)_4$ in a blood, plasma or serum sample (claim 49) or a neonatal blood sample (claim 50) by TMS. As discussed at length in the preceding sections, none of the cited references disclose or suggest a method of screening subjects for Pompe disease by detecting $(Glc)_4$ by any method. Further, none of the references disclose or suggest that $(Glc)_4$ is a biomarker for Pompe disease in neonatal subjects. As noted above, the Lancet reference discusses urinary $(Glc)_4$ excretion in a pregnant woman but does not address $(Glc)_4$ accumulation in any fetal tissue or fluid. In addition, there is no teaching in the cited references to suggest that $(Glc)_4$ is present in neonates, in particular neonatal blood. As far as the Applicants are aware, it was not previously known that $(Glc)_4$ accumulates in blood. Further, it would not have been obvious that $(Glc)_4$ would accumulate in blood based on reports of urinary excretion of $(Glc)_4$. In summary, none of the cited references taken alone or in any combination suggest a method of screening a neonatal subject for Pompe disease by detecting $(Glc)_4$ using TMS or determining the concentration of $(Glc)_4$ in any biological sample, including blood, plasma or serum samples, using TMS.

In view of the discussion above, the subject matter of claims 39, 40, 49 and 50 is nonobvious over the cited references alone or in any combination, and Applicants respectfully request that the outstanding § 103 rejection be withdrawn.

G. Determining the Concentration of $(Glc)_4$ by TMS in a Neonatal Urine Sample.

Claim 51 stands rejected as unpatentable for obviousness over Lancet further in view of Millington et al. There is no teaching in the cited references to suggest that $(Glc)_4$ is present in neonates at all, much less excreted into the urine by neonates. Further, neither reference discloses or suggests a method of determining the concentration of $(Glc)_4$ using TMS. In the absence of such disclosure or suggestion, the subject matter of claim 51 is nonobvious over Lancet or Millington et al., alone or in combination, and Applicants respectfully request withdrawal of the present obviousness rejection over these references.

IV. Conclusion.

The concerns of the Examiner having been addressed in full, Applicants respectfully request withdrawal of all outstanding rejections and the issuance of a Notice of Allowance forthwith. The Examiner is encouraged to address any questions regarding the foregoing to the undersigned attorney, who may be reached at (919) 854-1400.

Respectfully submitted,

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